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TITLE: Use of the HSP 110 Peptide Binding Protein for the Development of New Breast Cancer Vaccines

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CONTRACTING ORGANIZATION: Health Research, Inc.
Buffalo, New York 14263-0001

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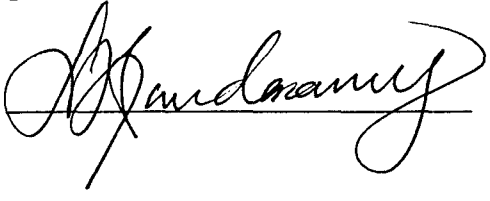

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13. ABSTRACT *(Maximum 200 words)*

Heat shock proteins have been examined in the last few years as anti-cancer vaccines. There are only a few major heat shock protein families in mammals, the most obvious being hsp28, hsp70, hsp90 and hsp110. To date, hsp70 and hsp90 family members have been the primary candidates for preparation of anti-tumor vaccines. Hsp110 has remained largely unstudied. Recently, hsp110 has been initially cloned and characterized in this laboratory. We have hypothesize in our application, based largely on sequence data, that hsp110 may be an excellent candidate for use as an anti-tumor, heat shock protein vaccine. The objective of our proposal was to test this hypothesis and examine peptides bound to hsp110. This first annual report describes the protocol for the purification of hsp110 from tumors and normal tissue. Moreover, it describes the initial studies of purified hsp110 in inhibiting the growth of a murine tumor model and in significantly extending survivability. These results demonstrate that hsp110 can be used as an anti-cancer vaccine.

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FOREWORD

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4. TABLE OF CONTENTS

5. INTRODUCTION

Devising means to stimulate an anti-tumor immune response represents a powerful approach to the control and elimination of systemic disease in breast and other forms of cancer. A novel approach to this has been proposed in the last few years. *This approach takes advantage of the promiscuous peptide binding properties of a small set of genetically conserved proteins known as heat shock proteins.* These molecular chaperones bind peptide chain and are involved in numerous protein folding, transport and assembly processes, and may even be involved in the antigen presentation pathway to MHC complexes. Purification of specific heat shock proteins from tumors also provides a plethora of associated tumor cell peptides, some of which are *immunogenic.* Use of these properties of specific heat shock proteins provides a general and simple approach in the preparation of anti-tumor vaccines has been demonstrated to produce an anti-tumor immune response in animals.

There are only a few major heat shock protein families in mammals, the most obvious being hsp28, hsp70, hsp90 and hsp110. To date, hsp70 and hsp90 family members have been the primary candidates for preparation of anti-tumor vaccines. Not all stress proteins function as vaccines and it can be expected that different ones may exhibit different activities. Curiously, hsp110 has remained, until recently, uncloned and largely unstudied. Recently, hsp110 has been initially cloned and characterized in this laboratory. We have hypothesize in our application, based largely on sequence data, that hsp110 may be an excellent candidate for use as an anti-tumor, heat shock protein vaccine. The objective of our proposal was to test this hypothesis and examine peptides bound to hsp110.

6. BODY

We have perfected an optimal purification scheme for hsp110 (Objective 1, Task A). In short, tumor or normal tissue are homogenized in hypotonic buffer and centrifuged at 100,000g. The supernatent is mixed with ConA-sepharose beads and the the unbound material separated on a column. This material is then applied to an ion exchange column (MonoQ). Bound proteins are eluted by a salt gradient (200-400 mM NaCl). The hsp110 fractions are then pooled, concentrated and loaded on a size exclusion column (Superose 6). Fractions are again collected and tested using anti-hsp110 antibody. This provides a homogenous hsp110 sample when examined by silver staining of gels.

In working out this purification protocol, it was observed that hsp110 exists in native form in a large complex of 400 kDa to 700 kDa. We have verified that this "hsp110 complex" also includes hsc70 and hsp28 and that the three actively interact (bind to one another) in situ. This suggests that these three chaperones form a chaperoning machine in cells. The chaperoning functions of this complex are being examined and a study of the vaccine potential of the complete complex is under consideration.

Next, we have tested the effectiveness of different doses of hsp110 as an anti-cancer vaccine, using Colon 26 tumors grown in Balb/c mice (as outlined in Objective 2,

Task A of application). This is a highly aggressive tumor system and was not listed as a model in the initial appication. We have chosen to add this model to our studies because of its aggressive nature and because we have gained considerable experience with it in other ongoing studies in the lab. In this study intradermal injections with hsp110 were performed two times, with innoculations separated by 10 days. Varying quantities of tumor derived hsp110 were used and hsp110 isolated from the livers of the same animals were used as a control (injections with Phosphate Buffered Saline/PBS as a control was also used). The data is shown in the Appendix in figure 1. It is clearly evident, using average tumor volume, that increasing quantities of hsp110 have increasing inhibitory effects on tumor growth rates. However, with this highly aggressive tumor model, mice were not cured. Nonetheless, the data indicate that hsp110 has specific antitumor effects. In comparison, we also examined hsc70 in this tumor model, which has been previously demonstrated to be a highly effective anti-cancer in other tumor models (1,2). Hsc70 slowed tumor growth but was not curative (data not shown).

Another manner in which such studies can be presented is to measure animal survival. While tumor growth provides average tumor volume data, it provides no direct insight into changes in life expectancy. Figure 2 presents this data for hsp110, again using this tumor model. In these studies, tumors are first established and then the animal is vaccinated. It is seen that tumor derived hsp110 has a significant impact on the life expectancy of mice, while PBS or liver (from the same animals) derived hsp110 have little or no effect.

Final studies are underway to examine the vaccine potential of hsp110 using the Meth A tumor system grown in Balb/c mice, as described in the application. This data is highly positive and the final results will be presented in the next report.

7. KEY OUTCOMES

The key outcomes from the above data are the purification scheme for hsp110 and the demonstration that hsp110 exhibits the clear ability to function in vivo as an anti-cancer vaccine when purified from the tumor.

8. REPORTABLE OUTCOMES

None in the first year of study directly related to the Statement of Work. However, a manuscript is in preparation describing the vaccine potential of hsp110. A second manuscript is close to submission, describing the purification protocol for hsp110 and the possible significance of the hsp110 complex described above ("Characterization of the native interactions of hsp110 with hsc70 and hsp28", Wang, Kazim, Repasky and Subjeck).

9. CONCLUSIONS

Hsp110 has anti-cancer vaccine activity.

10. REFERENCES

1. udono, H., and Srivastava, P.K. Heat Shock Protein 70-associated peptides elicit specific cancer immunity. Journal of Experimental Medicine. 178:1391-1396, 1993.

2. Tamura, Y., Peng, P., Liu, K., Daou, M., Srivastava, P. K. Immunotherapy of tumors with autologous tumor-derived heat shock protein preparations. Science 278: 117-120. 1997.

11. APPENDICES

Figures 1 and 2, attached.

Figure 1

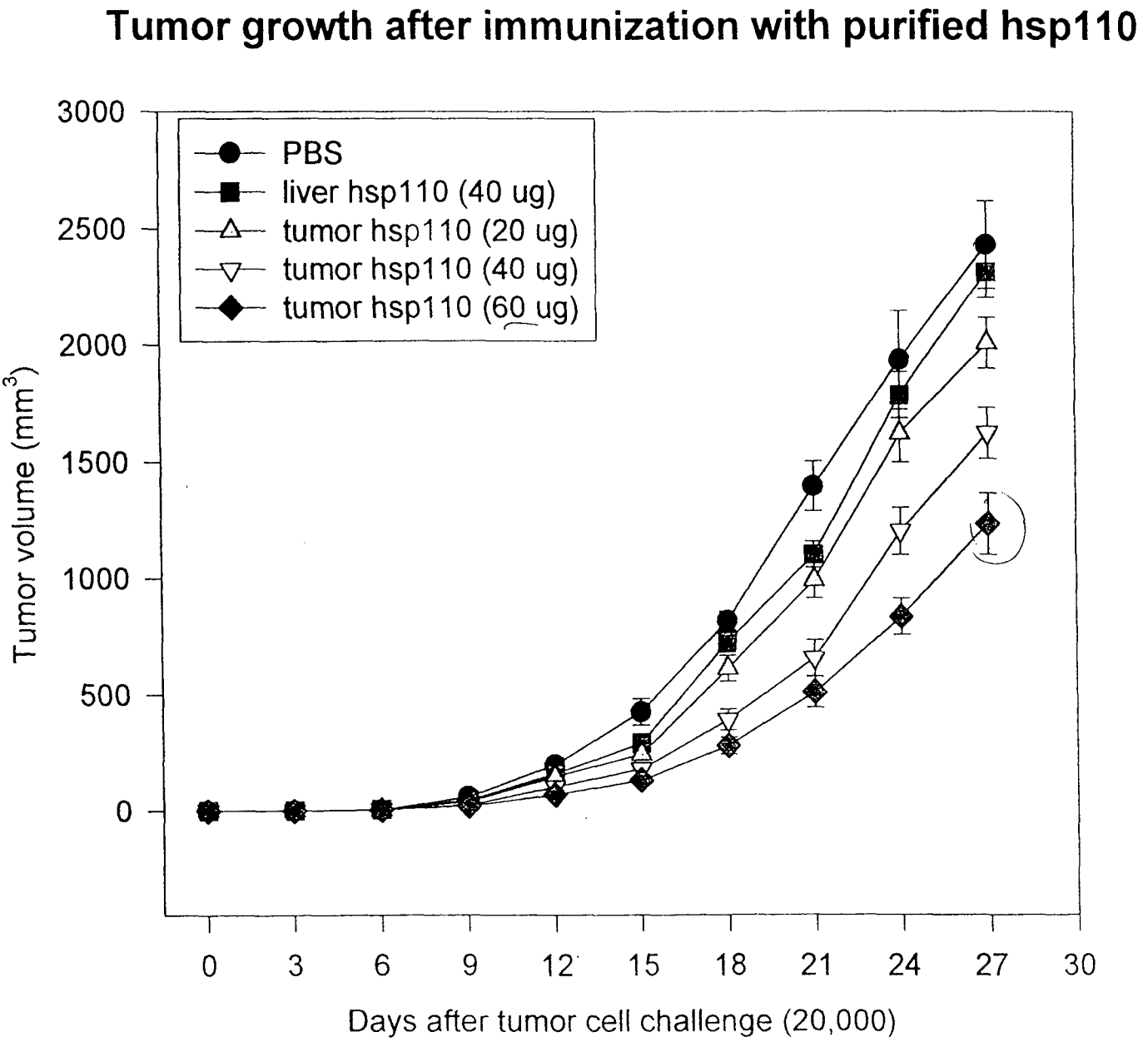
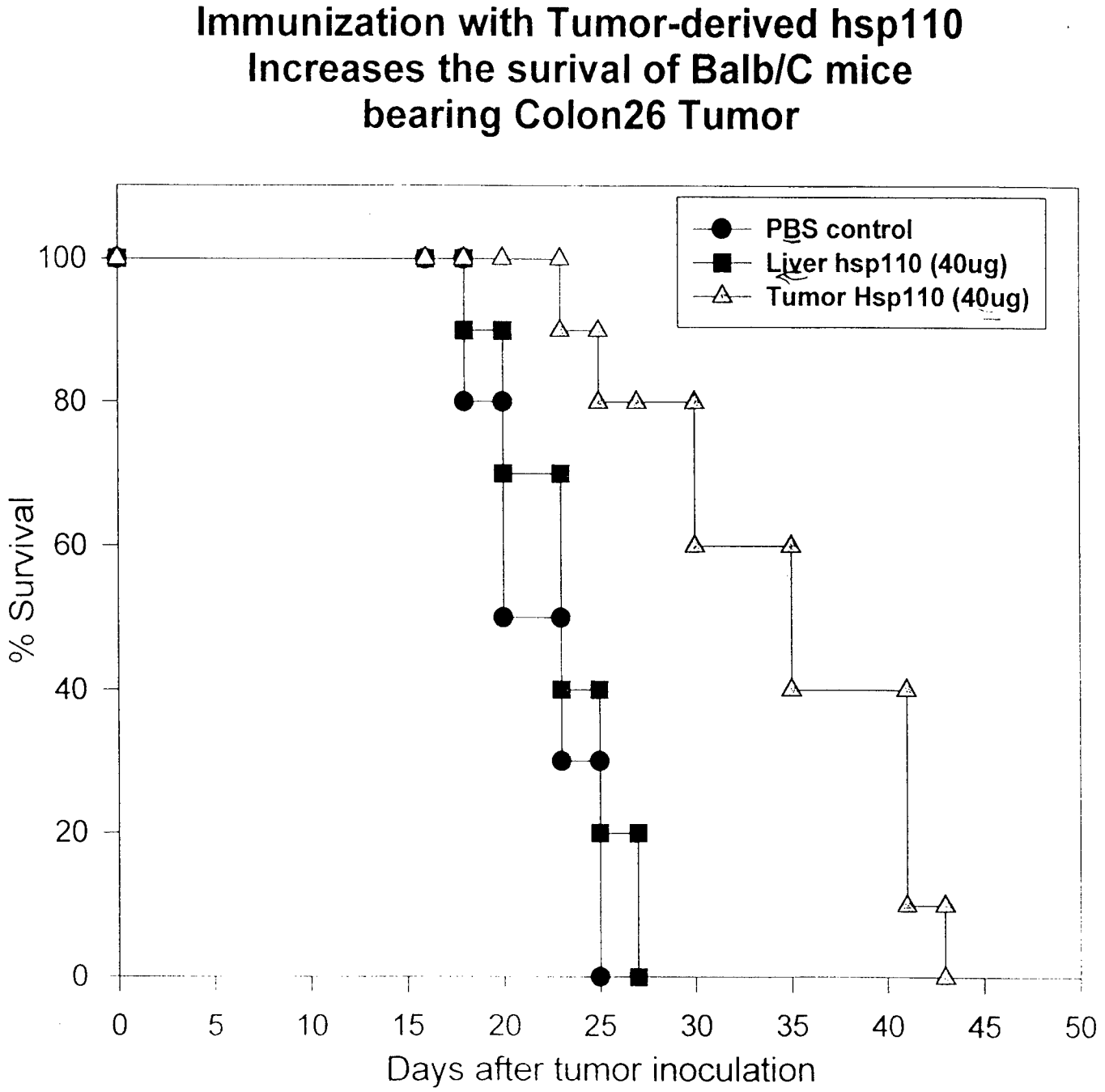

Tumor growth after immunization with purified hsp110
PBS
liver hsp110 (40 ug)
tumor hsp110 (20 ug)
tumor hsp110 (40 ug)
tumor hsp110 (60 ug)
Tumor volume (mm3)
3000
2500
2000
1500
1000
500
0
0
3
6
9
12
15
18
21
24
27
30
Days after tumor cell challenge (20,000)

Figure 2


Immunization with Tumor-derived hsp110
Increases the surival of Balb/C mice
bearing Colon26 Tumor
PBS control
Liver hsp110 (40ug)
Tumor Hsp110 (40ug)
% Survival
100
80
60
40
20
0
0
5
10
15
20
25
30
35
40
45
50
Days after tumor inoculation

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FOR THE COMMANDER:

PHYLLIS M. RINEHART
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